TECHNICAL PAPERS

Chloromycetin, a New Antibiotic From a Soil Actinomycete

JOHN EHRLICH, QUENTIN R. BARTZ, ROBERT M. SMITH, and DWIGHT A. JOSLYN

The Research Laboratories, Parke, Davis & Company, Detroit

PAUL R. BURKHOLDER

Osborn Botanical Laboratory, Yale University

Fom a soil sample collected in a mulched field near Caracas, Venezuela, a *Streptomyces* sp. was isolated.[1] Agar streak cultures were found to inhibit adjacent inocula of *Bacillus mycoides*,[1] *B. subtilis*,[1] *Mycobacterium tuberculosis* var. *hominis* (ATCC 607[1] and H37Rv[2]), *Staphylococcus aureus*,[1,3] *Streptococcus pyogenes*,[3] *Brucella abortus*,[3] *Escherichia coli*,[1,3] *Klebsiella pneumoniae*,[3] *Salmonella schottmuelleri*,[3] and *Shigella paradysenteriae* (Sonne).[3] When the organism was grown in liquid media in shaken flasks, filtrates of these submerged aerated cultures proved to possess marked antibacterial activity in broth-dilution assays against several gram-negative bacteria, notably *S. paradysenteriae* (Sonne), and indications of antirickettsial activity. From these filtrates a crystalline antibiotic has been isolated, for which the name Chloromycetin is proposed.

TABLE 1

Vol. medium	Container (capacity)	Yield (μg./ml.)
100 ml.	Erlenmeyer flask (500 ml.)	55
18 l.	Glass fermenter (30 l.)	85
100 gal.	Rotary aluminum drum (200 gal.)	49

The organism has been found to produce Chloromycetin in aerated submerged culture in various media. A satisfactory formula is: maltose, 1.0 per cent; casamino acids (Bacto), 0.5 per cent; distillers' solubles, 0.5 per cent; and sodium chloride, 0.5 per cent. Typical yields with this medium in various containers are given in Table 1. Potency is estimated turbidimetrically in terms of weight of crystalline material from a standard curve for 50 per cent inhibition of *S. paradysenteriae* (Sonne).

It was found that Chloromycetin could be concentrated and purified by extracting acidified culture filtrates with ethyl acetate, removing the solvent by distillation *in vacuo*, extracting the antibiotic with diethyl ether, chromatographing the ether solution over aluminum oxide, removing the solvent by evaporation, extracting the residue with water, extracting the aqueous solution with petroleum ether, and concentrating the aqueous solution. During the course of the concentration the antibiotic crystallized. After three recrystallizations from methylene dichloride, ethylene dichloride, and a mixture of diethyl ether and petroleum ether, colorless needles or elongated plates having the following properties were obtained: m.p., 149.7–150.7°C. (corrected); $(\alpha)_D^{25}$, − 25.5° (ethyl acetate)[4]; solubility in water at 25°C., about 2.5 mg./ml.; very soluble in methanol, ethanol, butanol, propylene glycol, and acetone; analysis: C, 41.11; H, 3.89; N, 8.60; Cl (nonionic), 21.71.[5]

Chloromycetin is a neutral compound and is unique in that it contains both nitrogen and nonionic chlorine. It is furthermore characterized by being stable at room temperature in aqueous solutions over the pH range of 2–9 for more than 24 hours, and in distilled water is unaffected by boiling for 5 hours.

The *in vitro* activity of the crystalline material against several bacteria is shown in Table 2. The test against *Br. abortus* was performed by dissolving varying amounts of the crystals in agar and streaking with *Br. abortus*.[3] *Myco. tuberculosis* was assayed by an end-point broth-dilution method.[2] The other organisms were tested by a turbidimetric method.[3]

TABLE 2

WEIGHT OF CRYSTALLINE CHLOROMYCETIN CAUSING INHIBITION OF TEST ORGANISM

Species	μg./ml.	Inhibition (%)
Brucella abortus	2.0	100
Escherichia coli	0.33	50
Klebsiella pneumoniae	0.33	50
Mycobacterium tuberculosis (H37Rv)	12.5	100
Proteus sp.	0.33	50
Salmonella schottmuelleri	0.33	50
Shigella paradysenteriae (Sonne)	0.2	50
Staphylococcus aureus	1.0	50

The crystalline material showed marked chemotherapeutic activity against *Rickettsia prowazeki* in screening tests using chick embryos[6] and against a number of rickettsiae and one virus when tested in embryonated eggs or in mice (*1*).

The intravenous LD_{50} for 20-gram mice is 3.0 mg./mouse. In contrast to streptomycin, Chloromycetin appears to be well absorbed when administered orally to mice and dogs.[7]

Detailed accounts of these studies are in preparation.

Reference

1. SMADEL, J. E., and JACKSON, E. B. *Science*, 1947, **106**, 418.

[1] In Paul R. Burkholder's laboratory, Yale University.
[2] In Guy P. Youmans' laboratory, Northwestern University.
[3] In the Research Laboratories, Parke, Davis & Company.
[4] Determined by Robert L. Harris, Parke, Davis & Company.
[5] Determined by A. W. Spang, Parke, Davis & Company.
[6] Determined by I. W. McLean, Jr., Parke, Davis & Company.
[7] Determined by O. M. Gruhzit, Parke, Davis & Company.